PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 22.11.2001
Applicant's or agent's file reference ICOY/P23294PC	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03196	18/08/2000	20/08/1999

Applicant
IMPERIAL COLLEGE INNOVATIONS LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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Form PCT/IPEA/416 (July 1992)

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
ICOY/P23294PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03196	18/08/2000	20/08/1999

International Patent Classification (IPC) or national classification and IPC
C12N15/62

Applicant
IMPERIAL COLLEGE INNOVATIONS LIMITED et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 12 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/03/2001	22.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Celler, J Telephone No. +49 89 2399 7336

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03196

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-100	as originally filed	

Claims, No.:

12-25	as originally filed	
1-11,26-47	with telefax of	23/10/2001

Claims, pages:

101,102,105-108	with telefax of	23/10/2001

Drawings, sheets:

1/17-17/17	as originally filed

Sequence listing part of the description, pages:

1-9, filed with the letter of 11.10.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03196

the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-5(partly),6,7,8-16(partly),17-24,25-39(partly),40,41,42-45(partly),46,47.

because:

☒ the said international application, or the said claims Nos. 1-5,8-16,36 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 47 are so unclear that no meaningful opinion could be formed (*specify*):
see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-5(partly),6,7,8-16(partly),17-24,25-39(partly),40,41,42-45(partly),46,47(partly).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

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International application No. PCT/GB00/03196

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-5(partly)(N,IS), 8-16(partly)(N,IS), 25-35(partly)(N,IS,IA), 36(partly)(N,IS), 37-39(partly)(N,IS,IA), 42-45(partly)(N,IS;IA).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3(partly)
	No:	Claims	2,4,12-15,25,30,32,34,36-39(all partly)
Inventive step (IS)	Yes:	Claims	3(partly)
	No:	Claims	1,5,8-11,16,26-29,31,33,35,42-45(all partly)
Industrial applicability (IA)	Yes:	Claims	25-35,37-39,42-45(all partly)
	No:	Claims	

2. Citations and explanations
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03196

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB00/03196

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Due to the lack of unity of the present international application (see International Search Report and Re Item IV), the ISR was established only for the subject matter of Claims 1 - 5 (partly), 8 - 16 (partly), 25 - 39 (partly), 42 - 45 (partly) and 47 (partly). **As according to the provision of Rule 66.1(e) PCT claims relating to inventions in respect of which no ISR has been established need not be the subject of International Preliminary Examination, the present International Preliminary Examination Report is formulated only in respect of Claims 1 - 5 (partly), 8 - 16 (partly), 25 - 39 (partly), 42 - 45 (partly) and 47 (partly).**

2. Claim 47 is so unclear (Art. 6 PCT) that formulation of a meaningful opinion on novelty, inventive step and the industrial applicability of the subject matter for which protection is sought was not possible. Whereas the subject matter must be clearly defined in claims, said claim does not identify any technical feature of the invention that would allow a skilled person to distinguish the method, use, etc. of the claim from any other method, use, etc. already known in the art. Furthermore, it is not clear to which part of the application the expression "as herein disclosed" refers to. According to the provisions of Art. 6 PCT, the invention must be clearly defined in claims, **said reference and the lack of any technical features offend that requirement to such an extend that formulation of an objective and meaningful opinion is not possible**.

3. Claims 1 - 5, 8 - 16 and 36 relate to subject matter considered by this Authority to be covered by the provisions of **Rule 67.1(iv) PCT**. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject matter of these claims(Article 34(4)(a)(i) PCT).
   For the assessment of the present Claims 1 - 5, 8 - 16 and 36 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical

treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment. **However when acting as the International Preliminary Examination Authority (IPEA), EPO conducts the examination according to the provisions of Articles and Rules of PCT and not those of EPC. Consequently, the guidelines for examination under EPC also do not apply for the purpose of formulating IPER.**

Re Item IV

Lack of unity of invention

This Authority acting as IPEA sustains the opinion given as International Search Authority (ISA), indicated in the ISR that the present application refers to four inventions.

The first-mentioned invention is the subject matter of Claims 1 - 5 (partly), 8 - 16 (partly), 25 - 39 (partly), 42 - 45 (partly) and 47 (partly), which are directed towards forms of SNARE molecules resistant to cleavage by clostridial toxins, their uses and polynucleotides coding therefor.

The second invention is the subject matter of Claims 1 - 18 (partly), 25 - 39 (partly) and 42 - 47 (partly), which are directed towards the forms of SNARE molecules capable of blocking the proteolytic activity of clostridial toxins, their uses and polynucleotides coding therefor.

The third invention is the subject matter of Claims 25 - 28 (partly), 30 - 39 (partly), 42 - 44 (partly) and 46 - 47 (partly), which are directed towards forms of SNARE molecules capable of inhibiting SNARE-mediated exocytosis, their uses and polynucleotides coding therefor.

Finally, the fourth invention is the subject matter of Claims 26 - 28 (partly), 30 (partly), 33 - 35 (partly), 37 - 39 (partly), 40, 41, 42 - 44 (partly) and 47 (partly), which are directed towards a gene therapy delivery system based on proteolytically inactive form of clostridial toxin.

Irrespective of the opinion regarding unity of the application at the

present examination stage, present IPER is formulated only in relation to those parts of the international application in respect to which the ISR has been established, because according to the provisions of Rule 66.1(e) PCT, those parts of the application in respect to which no ISR has been established, need not be the subject of International Preliminary Examination.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present IPER is concerned with those parts of the international application that relate to what has been first indicated in Re Item IV and constitutes the first invention mentioned in present claims, i.e. the method of treating a patient suffering from poisoning or at risk of poisoning by clostridial toxin, use of SNARE in treatment of such a patient as above, a method of the inhibition of exocytosis in a cell, all based on forms of **SNARE molecules resistant to cleavage by clostridial toxins**, said molecules themselves and polynucleotides coding therefor, the method of making such SNARE molecules and corresponding expression constructs, including gene therapy constructs and pharmaceutical compositions as well as kits.

2. Reference is made to the following documents:

D1: GONELLE-GISPERT C., HALBAN, P. A., NIEMANN H., PALMER M., CATSICAS S., SADOUL K.: 'SNAP-25a and -25b isoforms are both expressed in insulin-secreting cells and can function in insulin secretion.' BIOCHEMISTRY JOURNAL, vol. 339, 1 April 1999 (1999-04-01), pages 159-165, XP002159203

D2: SADOUL K., BERGER A., NIEMANN H., WELLER U., ROCHE P.A., KLIP A., TRIMBLE W.S., REGAZZI R., CATSICAS S., HALBAN P.A:: 'SNAP-23 is not cleaved by botulinum neurotoxin E and can replace SNAP-25 in the process of insulin secretion' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 52, 26 December 1997

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET International application No. PCT/GB00/03196

(1997-12-26), pages 33023-33027, XP002159204

D3: BRUNSD D., ENGERS S., YANG C., OSSIG R., JEROMIN A., JAHN R.: 'Inhibition of transmiter release correlated with the proteolytic activity of tetanus toxin and botulinum toxin A in individual cultured synapsys of Hirudo medicinalis' JOURNAL OF NEUROSCIENCE, vol. 17, no. 6, 15 March 1997 (1997-03-15), pages 1898-1910, XP002159205

D4: WO 95 32738 A (ALERGEN) 7 December 1995 (1995-12-07) cited in the application

3.1 D1 discloses SNAP-25 isoforms that have been made resistant to BoNT/E cleavage by means of sight-directed mutagenesis (e.g. p.163, "Production of BoNT/E-resistant SNAP-25 isoforms"). To obtain the toxin-resistant, mutant isoforms the sequence around the BoNT/E cleavage site was changed (e.g. p. 159, "Abstract"). The generated isoforms were transfected into cells prior to treatment with the toxin (e.g. p. 162, "Functional role of SNAP-25 isoforms of insulin secretion"). The mutants did not prevent the toxin cleavage of the endogenous SNAP-25, therefore were not inhibitory towards the endopeptidase activity of the toxin. However, they were able to **functionally replace** in exocytosis the endogenous, wild-type SNAP-25. D2 discloses a similar, functional replacement of the endogenous SNAP-25 with naturally occurring form of SNAP-23 which is not cleavable by BoNT/E. **It is well known to those skilled in the art that clostridial poisoning, including botulism and tetanus, involves the proteolytic activity of the corresponding toxins and results from the inhibition of neurotransmitter release. Such a state of the art is also acknowledged in the present description. Consequently, the information provided in D1 and D2 would immediately appear to a skilled person relevant as a way of overcoming neurotransmitter release in poisoned cell, also cells within the body of a patient. Especially so in light of the teaching in D1 (page 159, first column, "Introduction") that VAMP-2 is essential for regulated release in endocrine cells the same as in neurons.** Thus a method of reversing the inhibition of exocytosis according to Claims 4,12 and 36 is implicit

in the disclosure of the prior art and not new in the sense of Art 33(2) PCT. Furthermore, mutant SNAP-25 of D1 is a **variant** of SNAP-25, **or of any fragment thereof and therefore, as such**, falls under the scope of Claim 30 and renders it not novel.

3.2 Consequently, the mutant SNAP-25 of D1 or SNAP-23 of D2 also appear to be suitable for use in medicine or for use in manufacture of medicament for the treatment of a patient suffering from poisoning or at risk of poisoning by clostridial toxin, which, in turn renders the subject matter of Claims 2, 13 - 15, 25 and 32 not new (Art. 33(2) PCT). D1 discloses also the existence of the nucleic acid encoding the mutant SNAP-25 (e.g. p.160, "Mutagenesis of SNAP-25 isoforms"), which renders Claims 34, 37, 38 and 39 not new (Art. 33(2) PCT).

4.1 From D3 a skilled person would know that tetanus toxin proteolyses synaptobrevin and that synaptobrevin, SNAP-25 and syntaxin are targets of clostridial toxins (e.g. p. 1898, "Abstract" and "Introduction" as well as references therein). Furthermore, it is demonstrated in D3 that the amino acid residues on both sides of the toxin cleavage site in SNARES are responsible for the degree of cleavability and that the toxin resistant forms of these molecules can be created (e.g. p. 1902, "Leech SNAP-25 is resistant to cleavage by BoNT/A" also Fig. 2B). For example, particular importance of residue Q197 of mammalian SNAP-25 is made evident (Fig. 2B). The cleavage resistant forms of SNARE molecules were demonstrated to be able to render the synaptic transmission toxin insensitive (e.g. p. 1908, right-hand-side column). The method of restoring SNARE based vesicle fusion of D3 has all of the essential technical features of the method of treatment of Claim 1. The difference between the method of Claim 1 and the disclosure of D3, seen as the closest prior art, lies in that the latter does not explicitly proclaim the method of treatment of a patient. However, In light of the information in D2 a skilled person faced with the problem of treating botulinum or tetanus toxin poisoning would see as an obvious choice for candidate treatment the toxin resistant forms of SNARE molecules. In consequence, the method of treatment of Claim 1 is not regarded as inventive in the sense of Art. 33(3) PCT. From D3 It is also obvious that the SNARE could be syntaxin or synaptobrevin. Therefore, Claim 5 also lacks the presence of inventive step.

4.2 Furthermore, the choice of residues surrounding the toxin cleavage site becomes

obvious in light of D3. The importance for toxin resistance of the residues located in the proximity to the cleavage site is further underscored by D2 (e.g. p. 33024 "SNAP-23 is resistant to cleavage by BoNT/E"), which renders Claims 10, 11, 29, 31 and 33 not inventive (Art. 33(3) PCT). Also the fact that tetanus and botulinum toxin A , B, C1 as well as others can be involved appears obvious. In consequence Claims 8 and 9 lack inventive step.

4.3 D3 teaches a skilled person that specific forms of SNARE molecules are resistant to some, bu not all, specific types of toxins (e.g. p. 19088, right-hand-side column). In light of that, the need for identification of the type of toxin from poisoning with which a patient is suffering becomes a matter of obviousness, which renders Claims 16 and 45 not inventive (Art. 33(3) PCT).

4.4 Moreover, as the subject matter of Claim 30 is not new and that of Claims 29 and 31 is not inventive, the method of making a polypeptide according to Claim 35 also lacks inventive step (Art. 33(3) PCT).

4.5 D4 discloses (active or inactive) clostridial toxin, which is able to enter the target nerve cell and facilitate the introduction of a drug or any other biologically active molecule (e.g. p. 2, "Summery of the invention"). In light of the teaching of D4 It is obvious to use any inactive form of the toxin as the targeting moiety to bring any molecule attached to it into a cell that can be entered by the toxin moiety.
The approach of D4, seen as the closest prior art, differs from the subject matter of Claims 26, 27, 28 and 42 in that the part attached to the targeting moiety derived from the toxin, is a form of SNARE molecule or a gene therapy delivery systems. The forms of SNARE molecules and gene therapy delivery systems are however particular embodiments of the general approach of D4. Consequently, as the SNARE molecules of Claim 26 are not new (see above) and a gene therapy construct of Claim 39 is not new (see above) the subject matter of Claims 26, 27, 28 and 42 falls in its entirety under the scope of teaching of D4 and therefore lacks the presence of inventive step. Further introduction of technical features well known in the art, such as cell-specific promoter or a pharmaceutically acceptable carriers does not render the subject matter of Claims 43 and 44 inventive (Art. 33(3) PCT).

4.6 As outlined in 3.1 above, D1 and D2 disclose the methods of reversing the inhibition of exocytosis caused by poisoning of cells with clostridial toxins, by supplying to the cells SNARE molecules which are resistant to proteolysis by said clostridial toxin. The methods of reversing the inhibition of D1 and D2 differ from the method of present Claim 3 in that the resistant to proteolysis SNARE is supplied before contact of said cells with clostridial toxin. The method of Claim 3 requires that said SNARE is provided not before contact of the cells with the toxin. The demonstration in the present application of the ability of the cleavage resistant SNARE molecules to rescue intact pre-poisoned cells appears to offer an advantage in that the treatment with such SNARE molecules of a patient who came in contact with clostridial toxin some time earlier appears now more plausible. Therefore the subject matter of Claim 3 is new and inventive in the sense of Art. 33.2 and 33.3 PCT. It should be noted that as long as claims dependent directly and indirectly on Claim 3 remain not so restricted as to limit the scope of protection sought to exclude the methods known and obvious in light of the cited prior art, the acknowledgment of novelty and/or inventive step is not possible for said dependent claims.

Re Item VIII

Certain observations on the international application

The expressions "variant", "fragment" and "derivative" in Claims 30 and 37 render the scope of the subject matte unclear (Art. 6 PCT). It is not clear to a skilled person which and how many residues of the molecule can be changed and/or deleted or added as well as what other modification can be carried out so that the molecule is still regarded as a "variant", "fragment" or "derivative" of SNARE. For example, from an extreme view point an oligonucleotide 2- or 3-nucleotide long or peptide 2- or 3-amino acid long, can be regarded also as a "variant", "fragment" or "derivative" of SNARE. In consequence there is a large number of molecules known from the prior art which foll under the scope of said claims which makes acknowledgement of novelty not possible.

CLAIMS

1. A method of treating a patient suffering from poisoning or at risk of poisoning by a clostridial toxin wherein a SNARE (soluble (*N*-ethylmaleimide-sensitive fusion protein)-attachment protein receptor) is supplied to a cell of the patient, wherein the SNARE is resistant to proteolysis by the said clostridial toxin (toxin-resistant SNARE) and/or is capable of inhibiting the clostridial toxin (toxin-inhibitory SNARE).

2. The use of a SNARE (soluble (*N*-ethylmaleimide-sensitive fusion protein)-attachment protein receptor), or of a recombinant polynucleotide capable of expressing the said SNARE in the manufacture of a medicament for the treatment of a patient suffering from poisoning or at risk of poisoning by the said clostridial toxin, wherein the SNARE is resistant to proteolysis by the said clostridial toxin (toxin-resistant SNARE) and/or is capable of inhibiting the clostridial toxin (toxin-inhibitory SNARE).

3. A method of reversing the inhibition of exocytosis in a cell caused by contact of a clostridial toxin with the said cell, wherein a SNARE is supplied to the said cell not before contact of the said clostridial toxin with the said cell, wherein the SNARE is resistant to proteolysis by the said clostridial toxin (toxin-resistant SNARE) and/or is capable of inhibiting the clostridial toxin (toxin-inhibitory SNARE).

4. The method or use of any one of claims 1 to 3 wherein the said SNARE that is resistant to proteolysis by the said clostridial toxin is synaptosomal-associated polypeptide of 25 kDA (SNAP-25) that is resistant to proteolysis by the said clostridial toxin.

5. The method or use of any one of claims 1 to 3 wherein the said SNARE that is resistant to proteolysis by the said clostridial toxin is syntaxin 1 or synaptobrevin that is resistant to proteolysis by the said clostridial toxin.

6. The method of any of the preceding claims wherein the SNARE that is capable of inhibiting the clostridial toxin (toxin-inhibitory SNARE) is synaptosomal-associated polypeptide of 25 kDA (SNAP-25) that is capable of inhibiting the clostridial toxin.

7. The method of any of the preceding claims wherein the SNARE that is capable of inhibiting the clostridial toxin (toxin-inhibitory SNARE) is a SNARE in which the residue immediately N-terminal to the clostridial toxin cleavage site is replaced by a cysteine residue.

8. The method or use of any of claims 1 to 4, 6 to 7 wherein the said clostridial toxin is botulinum toxin A (BoNT/A).

9. The method or use of claim 4 wherein the said SNARE is resistant to proteolysis by botulinum toxins A, B and C1 (BoNT/A, BoNT/B and BoNT/C1).

10. The method or use of claim 8 or 9 when dependent on claim 4 wherein the said SNARE is a variant of SNAP-25 in which the residue equivalent to residue 197 and/or the residue equivalent to residue 198 of full length SNAP-25 are replaced by a residue other than ~~Gln~~ Q or a residue other than ~~Arg~~ R, respectively.

11. The method or use of claim 10 wherein the residue equivalent to R198 of full length human SNAP-25 is replaced by a residue other than R, ~~for example~~ preferably A, T, K, H or W and the residue equivalent to residue Q197 of full length SNAP-25 is Q or is replaced, ~~for example~~ preferably by A, K or W.

26. A molecule which comprises a SNARE polypeptide or toxin-resistant or toxin-inhibitory SNARE polypeptide or inhibitory SNARE polypeptide as defined in any of the preceding claims and a further portion.

27. The molecule of claim 26 wherein the said further portion is capable of promoting cellular uptake of the molecule or the said SNARE polypeptide or toxin-resistant or toxin-inhibitory SNARE polypeptide or inhibitory SNARE polypeptide.

28. The molecule of claim 26 or 27 wherein the said further portion is an inactive clostridial neurotoxin having specificity for a target nerve cell.

29. A polypeptide that is a variant, fragment, derivative or fusion of SNAP-25 that is resistant to cleavage by BoNT/A or is capable of inhibiting BoNT/A wherein (1) the residue equivalent to residue Q197 of full length SNAP-25 is replaced by A or W and the said fragment is at least 18 amino acids in length or (2) the residue equivalent to residue R198 of full length SNAP-25 is replaced by H or W, and the said fragment is at least 18 amino acids in length or (3) the residue equivalent to residue Q197 of full length SNAP-25 is replaced by A, K or W and the residue equivalent to R198 of full length SNAP-25 is replaced by a residue other than R, ~~for example~~ preferably A, T, K, H or W or (4) the residue equivalent to residue R198 of full length SNAP-25 is replaced by a residue other than R, ~~for example~~ preferably A, T, K, H or W wherein residues equivalent to one or more of amino acids 203 to 206 are not present or (5) the polypeptide is also resistant to cleavage by BoNT/E and BoNT/C or (6) the residue equivalent to residue Q197 of full length SNAP-25 is replaced by C.

30. A polypeptide consisting of residues identical to residues 1 to 198, 199, 200 or 201 of full length SNAP-25 or a variant thereof, or a fusion either thereof.

31. A SNARE in which the residue immediately N-terminal to a clostridial toxin cleavage site (~~for example~~ preferably BoNT/A cleavage site) is replaced by a cysteine residue.

32. A toxin-resistant or toxin-inhibitory SNARE or inhibitory SNARE as defined in any of the preceding claims or a molecule or polypeptide according to any one of claims 26 to 31 for use in medicine.

33. A nucleic acid encoding a polypeptide according to claim 29 or 31 or molecule according to claim 26, 27 or 28 or toxin-inhibitory SNARE as defined in any of the preceding claims.

34. A nucleic acid suitable for expressing a polypeptide according to claim 29 or 30 or 31 or molecule according to claim 26, 27 or 28 or toxin-inhibitory SNARE as defined in any of the preceding claims.

35. A method of making a polypeptide according to claim 29 or 30 or 31 or molecule according to claim 26, 27 or 28 or toxin-inhibitory SNARE as defined in any of the preceding claims, the method comprising culturing a host cell comprising a nucleic acid according to claim 34, and isolating said polypeptide or molecule from said host cell.

36. The method or use of any one of claims 1 to 24 wherein the said toxin-resistant or toxin-inhibitory SNARE or inhibitory SNARE is supplied to the said cell by means of expression of the said toxin-resistant or toxin-inhibitory SNARE or inhibitory SNARE in the cell.

37. A recombinant polynucleotide encoding a SNARE or a variant, fragment, derivative or fusion thereof for use in medicine.

38. A recombinant polynucleotide encoding a toxin-resistant or toxin-inhibitory SNARE or inhibitory SNARE as defined in any of the preceding claims or a nucleic acid according to claim 33 or 34 for use in medicine.

39. A gene therapy construct comprising a recombinant polynucleotide or nucleic acid as defined in claim 37 or 38.

40. A gene therapy delivery system comprising an inactive clostridial neurotoxin having specificity for a target nerve cell and a polynucleotide comprising a target nerve cell-specific promoter.

41. The gene therapy delivery system of claim 40 wherein the inactive clostridial neurotoxin has specificity for a cholinergic neuron and the target nerve cell-specific promoter is specific for a cholinergic neuron.

42. A gene therapy construct according to claim 39, further comprising an inactive clostridial neurotoxin having specificity for a target nerve cell.

43. A gene therapy construct according to claim 42 further comprising a target nerve cell-specific promoter.

44. A pharmaceutical formulation comprising a polypeptide as defined in claim 25, toxin-resistant SNARE or inhibitory SNARE, molecule or polypeptide as defined in claim 32 or polynucleotide as defined in claim 37 or 38 together with one or more acceptable carriers.

45. A kit of parts comprising (1) means for determining the type of clostridial, for preferably example botulinum, toxin from which a patient is suffering or means for determining

that a patient is suffering from a particular type of clostridal, preferably ~~for example~~ botulinum, toxin and (2) a toxin-resistant and/or toxin-inhibitory SNARE as defined in claim 1 or recombinant polynucleotide capable of expressing said toxin-resistant or toxin-inhibitory SNARE.

46. A kit of parts comprising (1) a toxin-resistant and/or toxin-inhibitory SNARE or recombinant polynucleotide capable of expressing said toxin-resistant SNARE as defined in claim 1 and (2) an inhibitor of the clostridial toxin to which the said toxin-resistant SNARE is resistant.

47. Any novel method of treatment, use, polypeptide, molecule or nucleic acid as herein disclosed.